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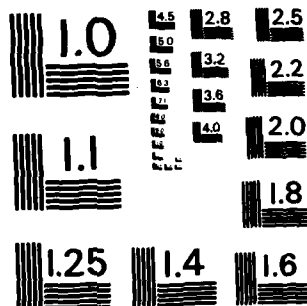
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PULMONARY ADAPTATION TO HIGH ALTITUDE

ANNUAL SUMMARY REPORT

Jerome A. Dempsey, Ph.D.

April, 1980

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The work accomplished during year 03 of the contract was aimed at defining the effects of hypoxia on various aspects of brain metabolism and their relationship to ventilatory regulation and to describing the effects of hypoxic acclimatization on ventilatory regulation during sleep. The following conclusions are warranted from the past year's work: (continued on back)			

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- a) Brain tissue pH (in 2 to 4 regions) is closely regulated during chronic hypoxia, with time-dependent changes in brain lactic acid forming the major contribution to this regulation. Cortex and brain stem lactic acid concentrations peak in acute hypoxia, decrease gradually with time, but show minimal reduction when acute normoxia is restored in the acclimatized animal. These data suggest that brain alkalosis is the key determinant of these lactate changes and that lactate production (and supposedly brain ECF acidification) is not positively correlated with ventilatory acclimatization.
- b) Progress was made with the ventricular-cisternal perfusion model in the awake goat with the emphasis on ventilatory effects.
- c) Significant effects of hypoxia on monoamine metabolism--level and turnover--were observed in both carotid body and the CNS, and significant adaptive effects were evident with duration of hypoxia.
- d) Pharmacologically induced changes in CNS serotonin metabolism had significant effects on normal ventilatory control, but manipulation of CNS catecholamines had no effect on ventilatory control or acclimatization.
- e) Breathing periodicity leading to marked HbO₂ desaturation was the primary finding during acute (4-24 hrs) hypoxia in humans. This instability was removed by acute normoxia and also disappeared with time in hypoxia (4 days). These trends toward a "stability" in breathing pattern during sleep were clearly the most profound effects on ventilatory control experienced during acclimatization.



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ANNUAL PROGRESS REPORT

(Year 03 - March 1, 1979 - April 1, 1980)

Work was carried out during the contract period according to four specific aims as originally outlined in our year 03 contract proposal. In general, our studies deal with ongoing concerns with the effects of chronic hypoxia on brain metabolism, its relationship to ventilatory acclimatization in various physiologic states and, in turn, the effects of anomalies in ventilatory drive on the process of acclimatization in general.

PROGRESS TO DATE

I. Brain Tissue pH and Metabolism.

Three categories of studies have been completed on this objective.

Technically, we have accomplished the following:

- a) significantly improved our fluorometric assays of muscle and brain tissue thereby permitting more accurate estimation of pH_i from the creatine phosphokinase equilibrium technique. In dog brain this technique compared favorably with the total CO_2 and DMO methods;
- b) determined that N_2O anesthesia was most appropriate for studies of brain metabolism (Musch et al., Respir. Physiol. 39: 121, 1980).
- c) devised optimum in vivo freezing techniques in rats for brain (using liquid N_2) and muscle (using Freon₂₂ cryoprobe) while maintaining arterial blood gases at awake levels.

Regional changes in brain pH_i in anesthetized dogs in hypoxemic hypocapnia and hypocapnia alone (simulating 4300 m altitude) were determined over 5 hour periods. Most brain regions showed an initial alkalinization (alkalinity) with a subsequent increase in lactate production and then return to normal pH_i by 5 hours. (The preliminary results on these data were reported in year 02 annual report. The study is now completed and submitted for publication).

Our most comprehensive study on this topic was conducted in rats over 7 days of simulated 4300 m (Musch et al., Fed. Proc. 39(3): 830, 1980). Most of those data are now completed and the results show: a) an initial rise in brain stem and cortex lactic acid (at 2 hrs) which returns toward but not to control levels over 7 days of continued hypoxia; b) an initial acidification of brain stem and cortex pH_i which also gradually returned toward normal with duration of hypoxia; and c) when normoxia was acutely restored (for 2 hrs) following 2 hours of hypoxic exposure, brain stem and cortex lactic acid concentration returned to normal control levels following 2 hours of hypoxia (and pH_i returned to normal) but remained unchanged (or fell < 10% and insignificantly) when normoxia was acutely returned in the acclimatized animal (i.e. after 1 or 2 days at 4300 m). (See data summary in Table 1).

Two major implications concerning both the role of cerebral fluid pH in ventilatory acclimatization and the mediation of brain lactate production in hypoxia may be drawn from this study. The recent work of Fenc1 et al. (1979) shows an acid brain ECF in chronic hypoxia, secondary to an increased production of brain lactic acid. If this changed tissue lactate production is the major source of brain ECF acidification, our data would confirm an acid ECF; but would also suggest that this acidity reached its peak in acute hypoxia and declined thereafter as ventilation rose with duration of hypoxia. Thus, these findings would not implicate an increasing brain ECF acidity in the mediation of time-dependent ventilatory acclimatization to hypoxia. These and previous findings taken together also reveal the complexity of cerebral fluid acid-base status in chronic hypoxia, i.e. an alkaline bulk CSF, an acid ISF and a normal intracellular pH.

Secondly, the change in brain lactate in hypoxia appears to be closely coupled with the PCO_2 change rather than hypoxemia, per se. For example, the decrease in lactate to normal when acute normoxia was applied during short-term hypoxia was accompanied by a complete return of $PaCO_2$ to normal, whereas in chronic hypoxia acute return to normoxia produced barely measurable changes in both PCO_2 and lactate. A testing of this hypothesis is underway whereby PCO_2 will be held normal during acute hypoxemia and returned all the way to normal when acute normoxia is applied in chronic hypoxia.

II. "Central" and Peripheral Chemosensitivity in Chronic Hypoxia.

Two types of studies were proposed for this problem, one dealing with changes in carotid body monoamines in hypoxia and the other using the Pappenheimer-Fenc1 model of ventricular-cisternal perfusion in the awake goat to test the responsiveness of "central" chemoreceptors. The first is near completion, while the second has only barely begun to yield usable data.

a) Carotid body monoamines in chronic hypoxia. A portion of this year was devoted to applying our high pressure liquid chromatography assays for monoamine analysis of the very small amounts of tissue available from rat carotid bodies. This analysis involved:

1. Addition of 10 picomoles of dihydroxybenzylamine (DHBA, a nonnatural monoamine, chemically similar to NE, DA and 5HT) to each pair of carotid bodies (removed within 5 minutes of sacrifice and stored in liquid nitrogen).
2. Homogenation in acid butanol and reextraction into 0.01 N HCl.
3. Separation by high performance liquid chromatography (HPLC) on a strong cation exchange resin period. NE, DHBA, DA and 5 HT peaks are detected electrochemically (see Fig. 1).

We have shown that peak height is proportional to molarity; however, the relative peak height depends on relative column retention extraction efficiencies and other variables. Therefore, standard curves (Fig. 2)

were constructed for the low levels of monoamines expected to be recovered from rat carotid bodies. The regression lines derived from these standard curves are used to correct unknown NE, DA and 5HT levels from carotid body extractions relative to the observed recovery of the 10 picomoles DHBA external standard added to each tissue.

Using this method we have obtained the following values in normoxic, control conditions (in picomoles/pair rat carotid bodies).

	<u>NE</u>	<u>DA</u>	<u>5HT</u>
$\bar{x} \pm \text{S.D. (N)}$	$21 \pm 7 (13)$	$16 \pm 8 (13)$	$8 \pm 5 (12)$

With the carotid body technique in hand we have measured the change in monoamine level and turnover (1 hr buildup following pargyline blockade) during acclimatization to chronic hypoxia. In preliminary studies two types of findings have emerged:

1. The levels of all 3 monoamines increased in hypoxia with NE and 5HT doubling and DA increasing to 4 times normal levels. Carotid body hypertrophy might explain some of these findings.
2. The turnover of DA appears to be elevated in acute (1 hr) hypoxia and the turnover of 5HT is increased at later times (5 hr through 7 days).

We believe these findings may have substantial functional significance in terms of ventilatory control and acclimatization, especially in view of the recent work in goats and ponies which has shown the importance of intact carotid bodies to ventilatory acclimatization (1,2). We are following up on these initial studies by pursuing two routes of inquiry:

- a. we are examining the ventilatory effects of drugs which alter monoamine metabolism. Uniquely, these drugs are applied directly onto the carotid bodies, and
 - b. have just begun using the goat model, before and after carotid body denervation, in our studies of 5HT effects on ventilation and ventilatory adaptation.
- b) Ventricular-cisternal perfusion. The primary purpose of adapting this procedure was to test the hypothesis that the ventilatory responsiveness to brain ECF $[H^+]$ and its interaction with other stimuli would have changed in the presence of other chronic and acute ventilatory stimuli, such as hypoxia and/or a changing tissue metabolic rate. For example, our preliminary data with steady-state ventricular infusion in awake rats indicated that in chronic hypoxia, these animals become non-responsive to large acute decreases in perfusate $[HCO_3^-]$

(and $[H^+]$). Our attempts over the past 18 months in the awake goat have only just now begun to yield usable data. In addition to our development of the surgery, measurement techniques and training of the animals for testing under various physiologic conditions, significant progress to date has included the following:

1. In 3 successful preparations we have determined the time course and magnitude of steady-state ventilatory response during ventricular-cisternal perfusion under conditions of normal air-breathing at rest, hyperoxia, CO_2 breathing, and (on one occasion) normal mild treadmill exercise;
2. we have also estimated transependymal flux (using Fenc1's technique and equations) under normal conditions in these animals, and
3. we have spent considerable time on two potentially serious problems that effect both the ventilatory response to a changing brain ECF $[H^+]$ and the estimation of transependymal flux. The first problem covers the change in ionized calcium which occurs when $[HCO_3^-]$ is changed in the perfusate solution (3) and the very high sensitivity shown to changes in calcium, per se, in the mock CSF perfusate--at least in anesthetized animals (4,5). We have begun to explore this problem in the awake goat preparation by measuring ionized calcium (3) with ion specific electrodes in all of our "inflow and outflow" CSF solutions; and will then determine the independent effect of this magnitude of change on the ventilatory response in the awake goat under the various physiologic conditions currently under study. The second problem concerns the significant errors inherent in the determination of transependymal flux when one assumes a value for the $[HCO_3^-]$ in freshly formed CSF (c_f). An error analysis shows, for example, that random differences of $\pm 5-10$ mEq/kg H_2O of the actual to assumed value for $c_f [HCO_3^-]$, per se, can be shown to change the corresponding calculation of CSF to ECF bicarbonate disequilibrium by $\pm 3-7$ mEq/kg*. As yet we don't have a solution to this problem, especially in view of the absence of adequate techniques for the quantitation of $c_f [HCO_3^-]$ in vivo. For the moment we have only tried various methods of plotting the ventricular-cisternal perfusion data in attempts to present a more realistic qualitative estimate of the factors affecting inflow vs. outflow $[HCO_3^-]$. This approach has helped us place the problem in perspective but, of course, the complicating unknown (of $c_f [HCO_3^-]$) remains. As indicated above, our studies in the near-future will focus specifically on the ventilatory effects of ventricular-cisternal perfusion in various physiologic states.

*It does not, in our view, seem likely that this type of error would discredit the findings (by Fenc1 et al., 1979) that chronic hypoxia produced a significant disequilibrium between CSF and ISF $[HCO_3^-]$ and $[H^+]$ in the direction of an acidosis in ECF. On the other hand, the technique at this time clearly cannot yield quantitation of the absolute state of CSF-ISF ionic equilibrium or its magnitude of change.

III. Role of CNS Neurotransmitters in Ventilatory Control and Acclimatization.

- a) Serotonin (5HT) depletion studies in CNS were near completion at the initiation of this contract year. They have now been completed and the results recently published in full in the Journal of Clinical Investigation (Olson, E.B., Jr., et al., J. Clin. Invest. 64: 689, 1979). These studies are summarized in Table 2 and strongly implicate 5HT in the CNS as an important inhibition to normoxic eupneic ventilation.

Follow-up studies are currently underway to focus more closely on central depletion of monoamines alone in the awake animal model. These include ventricular injection of 5HT in awake rats, infusion of 5HT in awake goats, and the systemic and central administration of 5HT and selected monoamine depleters in awake, carotid denervated goats.

- b) The role of catecholamine neurotransmitters has been extensively studied in the awake rat model both in normoxia and over the time-course of acclimatization to and deacclimatization from 4300 m altitude. Alpha-methyl-tyrosine (α MT) was the primary pharmacologic means of depleting norepinephrine levels in brain. Dose regimens were chosen which by I.P. administration produced an average brain NE depletion of 60% in one group of 35 rats or 80% in a second group of 14 rats. In neither group of animals were there ventilation or blood gases significantly different from control animals at any time during eupnea or during exposure to or the acute return from hypoxia.

Since α MT inhibits the synthesis of DA and NE, the lack of a change in breathing could be the reflection of opposing influences of DA, located in the CNS or in the carotid body, and central NE neurons. To test this possibility we administered the compound FLA-63 (bis [4-methyl-1-homopiperazinyl thiocarbonyl] disulfide, 25 mg/kg I.V.) to a group of 8 awake rats. This compound inhibits the enzyme dopamine- β -hydroxylase which converts DA to NE and has been shown to reduce brain NE levels *in vivo* without altering DA levels (Corrodi et al., Brain Res., 1970). We attained ~ 70% decrease in NE with a small increase in DA. As with α MT this treatment did not result in any change in breathing compared to a group of paired control animals. These studies lead to the working hypothesis that, at least in this experimental animal model, catecholamines appear to have very little, if any, effect on eupneic breathing or on the ventilatory acclimatization to or deacclimatization from short-term hypoxia. Some of these data have been reported in preliminary form (McCrimmon, Fed. Proc. 38: 1191, 1979). The final studies on this topic are near completion and will be submitted for publication in the near future.

- c) Topical application of 5HT to the dorsal aspect of the medulla and C5 region of the spinal cord has been achieved in paralyzed, anesthetized cats. The drug effects were localized by injections of small 10-50 λ doses of drug onto the desired area from a cannula which was visually located in the correct location. As with the peripheral 5-HTP treatment local application of either 5-HT or DMS-HT onto the medulla resulted in

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results are currently being expanded to larger groups of animals and to conditions of acute return to normoxia at the varying time points of exposure. In addition varying doses of blockers (6FT and α MT) in addition to pargyline are being used as additional indices of monoamine turnover.

Summary of Neurotransmitter Studies.

As we outlined in original proposal, the basic question of neurotransmitter metabolism and its role in ventilatory control is a new concept in this field. It is our philosophy that several approaches must be taken to a single question on this topic, i.e. several lines of confirmatory evidence are needed in the testing of any hypothesis. Hence, our major approach has been through the use of pharmacological manipulation of monoamines in the awake animal model with concomitant measurements of neurotransmitter levels and turnover and of the functional response in terms of ventilation, metabolic rate and blood gases. The more descriptive studies of hypoxic acclimatization effects on neurotransmitter metabolism and ventilation are necessary physiological approaches to complement any pharmacologic evidence. The third approach using local applications, neurophysiological evidence in anesthetized animals, carotid body denervated animals and Raphé nuclei lesioning stresses site of action. The progress toward reasonable hypotheses from this multiple-faceted approach is, of course, quite slow. Nonetheless we are pleased with our advances to date and can point, for example, to the cumulative, consistent evidence supporting a significant role for CNS serotonin in the control of eupnic ventilation. The implications of this monoamine in the process of hypoxic acclimatization also appears promising, but currently unconfirmed. The exact mode and mechanism of action of these monoamines must await more intensive investigation.

IV. Human Ventilatory Control During Sleep in Hypoxia.

Studies of ventilatory control during sleep in hypoxia--even of a purely descriptive variety--are sorely lacking with the few studies conducted to date consisting only of oximeter measurements of arterial HbO₂ saturation. We had two aims in our study:

- a) To quantitate the effect of duration of hypoxic exposure (over 4 days) on breathing pattern and volume, arterial blood gases and acid-base status and pulmonary gas exchange during various phases of sleep. To this end four healthy subjects were studied while awake and over 5 to 8 hours of sleep per night in a hypobaric chamber simulating 4300 m altitude. To better define the effects of acute hypoxia during sleep each subject was studied during sleep within 1 to 3 hours of exposure to 4300 m. These studies required our redesigning our chamber and measurement systems to obtain continuous monitoring of 8 physiological variables outside the chamber walls.
- b) Our second aim was to compare the effects of hypoxia exposure during sleep to other chronic ventilatory drives and depressants. This study

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rapid and complete phrenic inhibition in 4 cats. Recovery time was 3-7 hrs following 7-20 mg of 5HT or ~ 45 min following 5 mg DM5-HT. Extracellular recording from a medullary neuron firing with a respiratory periodicity indicated that this neuron was turned off by local application of DM5-HT. Results from local application at the spinal level have so far been less conclusive with a much longer time course (30 min to 1 hr) requires for inhibition to occur. The inhibition of phrenic nerve activity by central application or I.V. administration of 5-HTP were also shown to be unaffected by carotid sinus nerve and vagal denervation, but could be "overcome" by acute respiratory acidosis (breathing 10% CO₂ in O₂). These studies were recently reported in preliminary form (McCrimmon, D.R., et al., *Fed. Proc.* 39(3): 952, 1980). They confirm our previous studies with systemic administration of 5HT depletion in awake rats and point firmly to a "central" inhibitory action of serotonin under conditions of air-breathing eupnea.

Another quite different approach to exploring central actions of monoamines is also underway in the form of lesioning serotonin containing neurons in the dorsal, medial and caudal Raphé nuclei. To date we have accomplished the following:

1. Dorsal lesion produces a 25% depletion in hemisphere 5HT and no significant change in any other region and any other monoamine (NE and DA).
2. The medial lesion produces a 50% depletion of hemisphere 5HT and a 25% depletion of stem 5HT and no other significant changes.
3. The caudal lesion produces a 25% depletion of cervical spinal cord 5HT and no other significant changes.

Substantial weight loss in lesioned animals has seriously complicated interpretation of any ventilatory measurements to date. We are, however, extremely pleased with the specificity of the lesions on neurotransmitter metabolism in the various regions and are currently attempting various diet regimens to improve the stability of these animals.

- d) Effects of hypoxic acclimatization on CNS neurotransmitter level and turnover has been studied over the time-course of 7 days of simulated 4300 m altitude. To date we have studied 10 rats at each of 5 time points in hypoxia (1 and 5 hrs; 1, 4, and 7 days). Weight-matched controls were sacrificed in parallel with hypoxic animals. Assays were performed on brain hemispheres, hypothalamus and stem, and systemic blood, heart and duodenum were also assayed. To date the results show no effects of hypoxia on neurotransmitter level or turnover in systemic tissues or blood or in hypothalamus. In brain stem, turnover of NE was depressed at 1 and 5 hr and NE levels were elevated at 4 and 7 days of hypoxia. 5HT levels were unaffected but turnover was depressed at 1 and 5 hr hypoxia. Turnover of both NE and 5HT in brain stem returned to normal during acclimatization. These preliminary

consisted of increasing ventilatory drive over a week period using medroxyprogesterone acetate (as we previously described, Skatrud, J.B., et al., *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 44(6): 939-944, 1978) and decreasing ventilatory drive over a 2-day period via NaHCO_3 infusion (+ 7-8 mEq/l $\Delta[\text{HCO}_3^-]$). Once a steady-state of ventilatory response was achieved in each case measurements were made awake and during all phases of sleep. A great deal of data was generated in these studies which is currently being analyzed manually (our taping system will undergo computer analyses). However, we do have sufficient data at hand, particularly from blood gas analyses to present some preliminary conclusions and new insights into the nature of ventilatory control under conditions of hypoxia and chronic changes in ventilatory drive:

1. In normoxia normal subjects experience significant respiratory acidosis (+4 to 8 torr PaCO_2) during non-REM sleep. Long-term MPA administration produced a hyperventilation and hypocapnia which was identical during awake and non-REM sleep states. Thus the effects of chronic ventilatory stimulation (via MPA) or inhibition (by increased $[\text{HCO}_3^-]$) are purely additive with the effects of slow-wave sleep. If slow-wave sleep may be taken as a model of so-called primary "metabolic" control of ventilation in humans, then we could postulate: (a) that suprapontine influences have a significant effect on normal eupnea; (b) but that these forebrain influences do not have an interactive effect with chronic stimuli or inhibition, *i.e.* at least those represented by hormone administration or alkalosis.
2. Hypoxia, particularly in the short-term, produces unique effects on ventilatory control and stability during sleep. These effects were particularly profound during the initial 4-24 hours of hypoxia and consist of frequent periods of erratic, ventilatory periodicity, apnea followed by hyperventilation, profound HbO_2 desaturation (to < 60% HbO_2 on many occasions) and "paradoxical"-type breathing whereby rib-cage contribution to tidal volume actually became negative. This ventilatory instability during sleep in hypoxia was removed by acute normoxia or hyperoxia independent of their effect on total ventilation.
3. The effects of four days acclimatization to hypoxia included an increase in the degree of hyperventilation and hypercapnia both awake and asleep, but the most profound effects were on breathing periodicity and apnea. By the third and fourth days the great majority of the periodicity, repeated apneic periods and marked HbO_2 desaturation during sleep were either completely absent or greatly diminished from their frequency of occurrence in the initial hours or first night spent at 4300 m. The data analysis continues with particular attention to the factors which might better characterize the breathing periodicity in hypoxic sleep *i.e.* correlation with specific sleep stage, relative changes in rib-cage vs. abdominal contribution to tidal volume, and

the role of a reduced end-expiratory level (i.e. FRC) in causing the marked desaturation in hypoxic sleep, especially REM sleep. The effects on these parameters of acute hyperoxia and duration of hypoxia will be particularly helpful because both of these factors reduce or remove the periodicity.

V. Summary of Progress in Year 03.

In general we are pleased with our progress in the past year in that most of our aims as originally stated in the contract proposal have been realized. Our progress with the ventricular-cisternal perfusion technique fell far short of expectations. We are, however, highly optimistic at this point and expect that our newer techniques and considerations in this area may lead to an even better experimental model than the original. On the positive side, we emphasize the success of our studies on brain lactate and acid-base regulation in hypoxia, our newer findings in the area of neurotransmitter metabolism and ventilatory control and the comprehensive data produced by our initial studies of ventilatory control during sleep in hypoxia. Technical advances in our laboratory were many this past year including; our neurotransmitter assay procedures and applications, our upgrading of measurement capabilities during sleep and their application to chamber studies in man, our recent application of a laboratory computer to on-line analysis of much of our data and our development of a neurophysiology laboratory and its beginning application to studies of sites of action of neurotransmitters on ventilatory control.

VI. Publications - Contract Year 03 (March 1, 1979 - April 1, 1980).

a) Manuscripts Published.

1. Dempsey, J.A., H.V. Forster, G.E. Bisgard, L.W. Chosy, P.G. Hanson, A.L. Kiorpes, and D.A. Pelligrino. Role of cerebrospinal fluid [H⁺] in ventilatory deacclimatization from chronic hypoxia. J. Clin. Invest. 64: 199-205, 1979.
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3. Musch, T.I., D.A. Pelligrino, and J.A. Dempsey. Effects of prolonged N₂O and barbiturate anesthesia on brain metabolism and pH in the dog. Respir. Physiol. 39: 121-131, 1980.
4. Dempsey, J.A., D.A. Pelligrino, D. Aggarwal, and E.B. Olson, Jr. The brain's role in exercise hyperpnea. Med. Sci. Sports 11: 213-220, 1979.
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6. Dempsey, J.A. and H.V. Forster. Chronic adaptations of ventilatory control mechanisms. In: The Regulation of Breathing, edited by T. Hornbein and C. Lenfant (In press, 1980).
 7. Dempsey, J.A. and H.V. Forster. Mediation of ventilatory adaptation. Physiological Reviews (In press, 1980).
 8. Dempsey, J.A., E.H. Vidruk, and S.M. Mastenbrook. Pulmonary control systems in exercise. Federation Proc. 39: 1498-1505, 1980.
 9. Bateman, N., C. Smith, T. Musch and J. Dempsey. Problems with the gas-calibrated PCO₂ electrode. Respir. Physiol. (In press, 1980).
- b) Symposium Presentations and Proceedings Abstracts.
1. Dempsey, J.A. "Ventilatory Control at High Altitude", Keynote Address at the Hypoxia Symposium, Banff, Alberta, Canada, February, 1979.
 2. Dempsey, J.A. "Pulmonary Control Systems in Exercise", presented at FASEB Symposium on Physiological Control Systems in Exercise, Dallas, Texas, April, 1979.
 3. FASEB (Federation of American Societies for Experimental Biology), Abstracts, 1980.
 - (a) McCrimmon, D.R., and P.M. Lalley. Inhibition of respiratory neural discharge in cats by clonidine and 5-hydroxytryptophan. Federation Proc. 39(3): 952, 1980.
 - (b) Musch, T.I., N.T. Bateman, C.A. Smith and J.A. Dempsey. Lactate production in rat brain during acclimatization to hypoxia. Federation Proc. 39(3): 830, 1980.
 - (c) Smith, C.A., N.T. Bateman, T.I. Musch, and J.A. Dempsey. Problems with the gas-calibrated PCO₂ electrode. Federation Proc. 39(3): 1092, 1980.

VII. Military Significance.

A detailed discussion of this topic is contained in our original contract application. Briefly, our results in year 03 have the following relevance to the well-being and performance of the soldier at high altitudes.

a) Basic Mechanisms.

In addition to their potential importance as primary or secondary regulators of ventilation, the metabolic system (tyrosine and tryptophan hydroxylation) controlling neurotransmitter metabolism in neural tissue represents a critical control over CNS function in hypoxia. Our studies have, for the first time, begun to describe the key changes

in this metabolic system during acclimatization to hypoxia. They have shown profound initial disturbances in these metabolic systems and in acid-base status in brain. With further time in hypoxia these two critical metabolic systems adapt and achieve homeostasis. Clearly these metabolic systems must play a key role in the medical and physiological problems and their accompanying symptoms of impaired judgment and performance which ensue upon hypoxic exposures; indeed their ability to compensate with time might well represent the real physiological basis of hypoxic "adaptation."

- b) Immediate applications of our research to the performance of the soldier at high altitudes may be found in two areas. First, our animal work emphasizes both the use of animal models which mimic human adaptations in many critical assays and the employment of physiologic perturbations (i.e. degree and duration of hypoxia, acid-base derangement, etc.) which are highly realistic in terms of human endeavors. Secondly, our initial sleep studies point strongly to the ventilatory and gas exchange problems encountered in this state as a principal contribution to performance decrement at high altitudes. It is especially instructive that intermittent normoxia (via sample nasal administration of O₂) alleviated the breathing periodicity and marked O₂ desaturation during the first 24 hours at high altitude. Further detailed studies are needed to determine the full implications of intermittent O₂ during sleep (in this state of acclimatization) on the physiologic state, quality of sleep, rapidity of further acclimatization, etc. at high altitudes.

VIII. Facilities.

New additions in year 03 include:

- a) a hypobaric chamber facility now in operation and complete with capabilities for human and animal studies and for "out-of-chamber" monitoring for sleep studies,
- b) laboratory computer, and
- c) a fully-equipped neurophysiology laboratory.

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TABLE 1. Effects of hypobaric hypoxia (4300 m simulation) on rat brain stem and cortex lactic acid concentration and tissue pH.

	CONTROL NORMOXIA (n=14)	HYPOXIA 2 HRS (n=12)	HYPOXIA 2 HRS & RETURN TO NORMOXIA (2 HRS) (n=5)	HYPOXIA 24 HRS (n=6)	HYPOXIA 24 HRS & RETURN TO NORMOXIA (2 HRS) (n=6)	HYPOXIA 7 DAYS (n=7)
<u>LACTATE</u> (mm/kg wet wt)						
Cortex	2.32 \pm .12	3.73 \pm .24	2.51 \pm .11	3.93 \pm .20	3.47 \pm .28	3.94 \pm .34
Stem	1.91 \pm .11	4.29 \pm .47	2.06 \pm .17	3.61 \pm .47	2.91 \pm .10	3.95 \pm .56
<u>pH_i</u>						
Cortex	7.118 \pm .026	7.044 \pm .042	7.196 \pm .016	7.121 \pm .014	7.201 \pm .026	7.181 \pm .032
Stem	7.249 \pm .053	7.053 \pm .034	7.300 \pm .019	7.134 \pm .030	7.121 \pm .043	7.222 \pm .050
<u>ARTERIAL ACID-BASE STATUS</u>						
PCO ₂ (mmHg)	40.0 \pm .5	27.7 \pm .8	35.4 \pm 1.0	21.5 \pm .7	23.1 \pm 1.1	21.5 \pm .5
pH	7.404 \pm .006	7.484 \pm .013	7.382 \pm .012	7.424 \pm .0013	7.423 \pm .008	7.432 \pm .017
[HCO ₃ ⁻] (mEq/l)	24.5 \pm .2	20.6 \pm .8	20.7 \pm .8	14.0 \pm .7	14.8 \pm .6	14.2 \pm .5
HCT (%)	40	40	40	46	45	48
PaO ₂ (mmHg)	85 \pm 2	47 \pm 2	85 \pm 1	50 \pm 1	90 \pm 2	54 \pm 1

TABLE 2. Effects of pharmacologic blockade on ventilation and brain NE, DA and 5HT concentrations
[Mean \pm 95% confidence limits (number treated)].

	PaCO ₂ (mmHg)	pH _a	\dot{V}_E/\dot{V}_{O_2}	NE (nanograms per gram)	DA (nanograms per gram)	5HT (nanograms per gram)
OVERALL CONTROL (N=83)	39.9 \pm 0.3	7.435 \pm .002	30.2 \pm 0.8	791 \pm 37	891 \pm 54	627 \pm 31
	(change from carrier controls)			(percent depletion from carrier controls)		
RESERPINE	-0.8 \pm 3.4(6) [‡]	.04 \pm .02(6) [‡]	0.2 \pm 10.7(6)	-91 \pm 8(3) [‡]	-91 \pm 32(3) [‡]	-86 \pm 20(3) [‡]
FCPA	-7.2 \pm 0.8(30) [‡]	.04 \pm .01(25) [‡]	5.0 \pm 3.0(12) [‡]	-22 \pm 11(8) [‡]	4 \pm 16(8)	-76 \pm 15(6) [‡]
ANT	-0.3 \pm 2.1(5)	.02 \pm .03(5)	4.4 \pm 5.9(6)	-86 \pm 5(15) [‡]	-73 \pm 6(15) [‡]	63 \pm 14(15) [‡]
6-PT	-5.9 \pm 1.4(11) [‡]	.06 \pm .01(11) [‡]	4.0 \pm 3.6(10) [‡]	-2 \pm 6(5)	-4 \pm 9(5)	-48 \pm 10(5) [‡]
FCA	-5.4 \pm 1.1(8) [‡]	.02 \pm .03(8)	4.3 \pm 4.2(8) [‡]	-4 \pm 13(5)	-1 \pm 5(5)	-52 \pm 14(5) [‡]
5,7-DHT	2.1 \pm 1.5(10) [‡]	.01 \pm .01(10)	-0.9 \pm 2.7(10)	9 \pm 8(20) [‡]	-16 \pm 13(20) [‡]	-60 \pm 11(20) [‡]

* P < .05

‡ P < .01

(Summary of data
ventilation in awake

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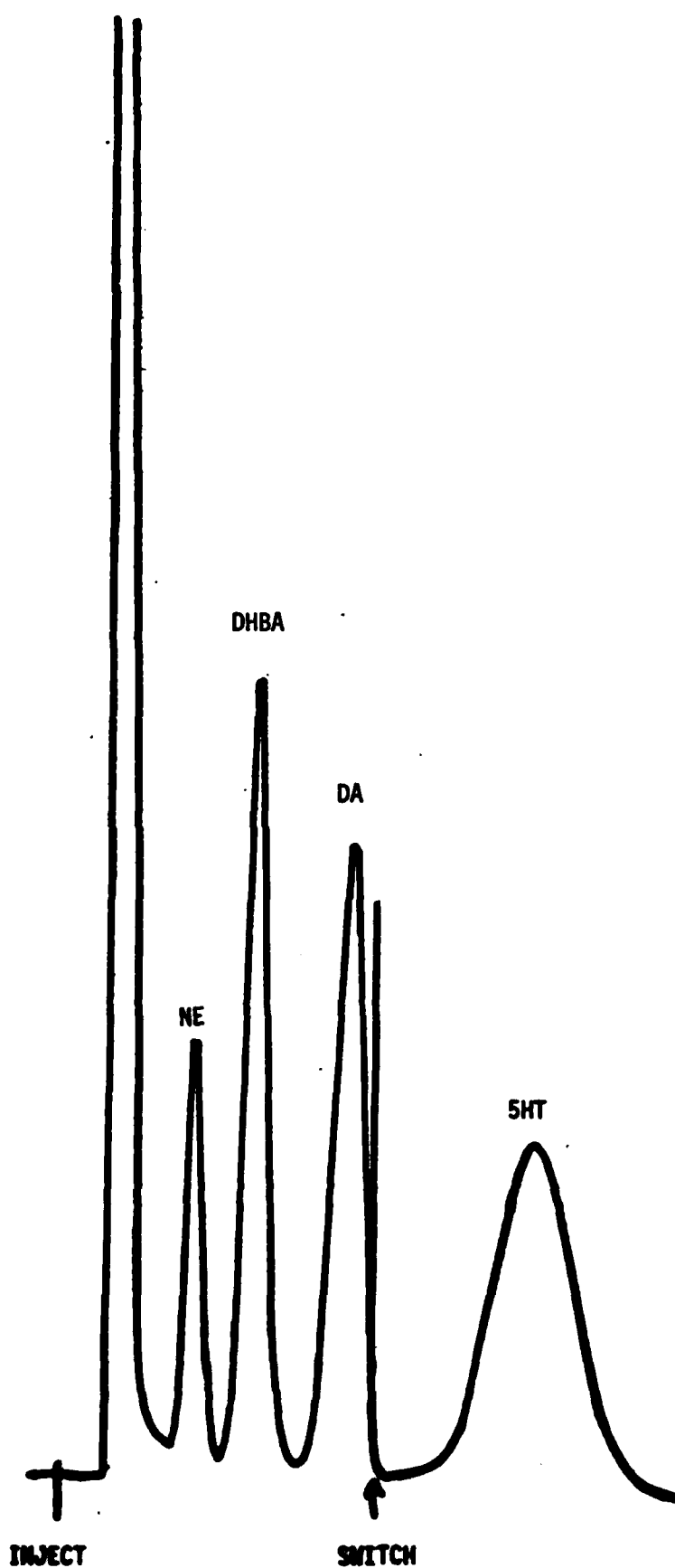


Fig. 1. HPLC electrochemical column detection profile showing relative retentions of all four monoamines.

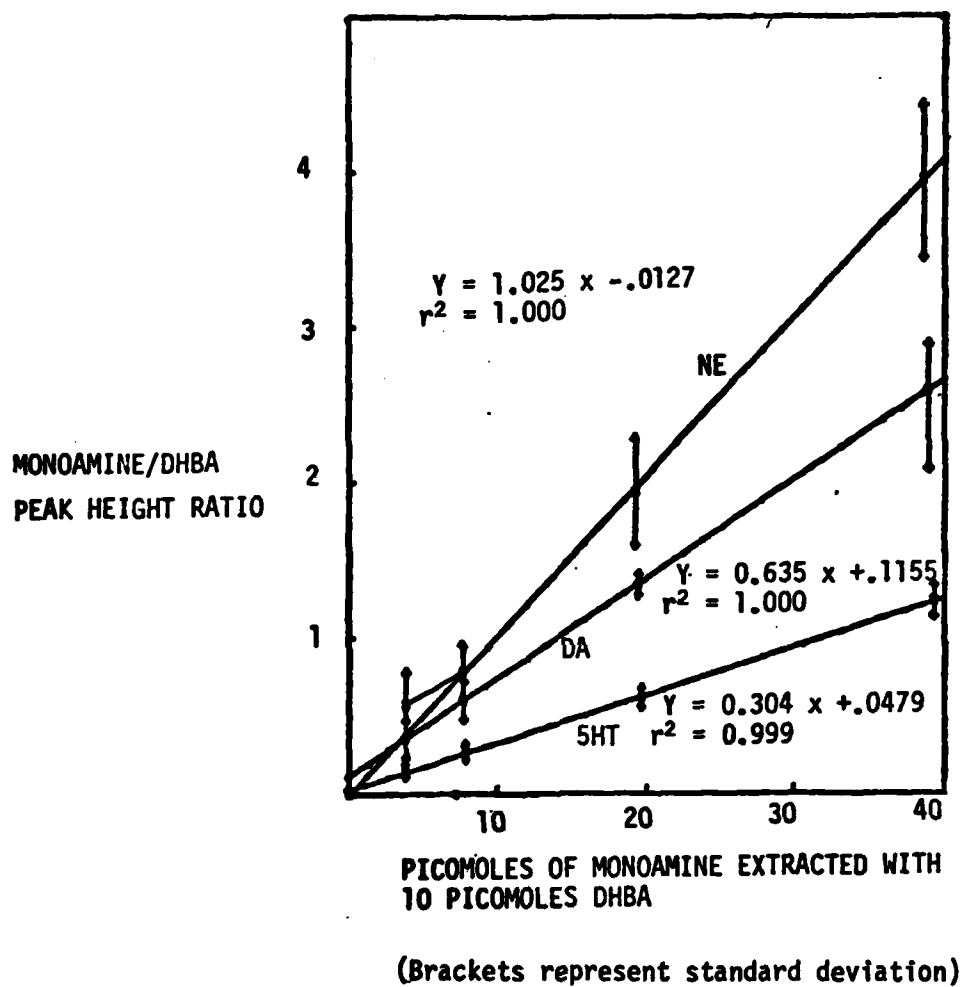


Fig. 2. Standard curves for monoamine assay in rat carotid bodies.

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